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Identification and Genetic Mapping of Genes for Hereditary Breast Cancer and Ovarian Cancer in Families Unlinked to BRCA1

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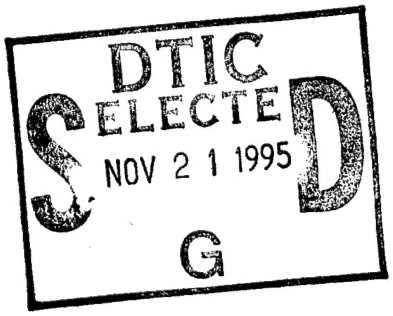

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13. ABSTRACT *(Maximum 200 words)*

Due to the successful completion of most of the goals of our original proposal through the localization of a second major breast cancer susceptibility locus, BRCA2, on chromosome 13q (Wooster et al., 1994), we have expanded the scope of the proposal in a number of significant ways. We now plan to use a set of existing and newly ascertained breast cancer families likely due to BRCA2 to better understand the cancer risks conferred by the gene and how other risk factors may interact with BRCA2 in determining risk. We also have begun studies designed to examine haplotype sharing for markers in the BRCA2 region in order to further localize the BRCA2 locus and eventually identify families which may derive from a common ancestral mutation. To this end we have developed 8 polymorphic genetic markers in the BRCA2 region; preliminary data suggest that there will be many distinct mutations in BRCA2. Based on penetrance analysis of two large BRCA2-linked families, BRCA2 carriers have an estimated risk for female breast cancer of 60% by age 50 and 80% by age 70, and a risk of male breast cancer by age 70 of 6%.

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David E Dolby 9/20/95

PI - Signature Date

TABLE OF CONTENTS

Annual Progress Report
DAMD17-94-J-4260

Annual Progress Report
Grant DAMD17-94-J-4260
September 20, 1995

Introduction

Since the submission of this proposal nearly two years ago, our knowledge of inherited breast cancer has increased greatly. The cloning of the BRCA1 (Miki et al., 1994) and the localization of a second major breast cancer susceptibility locus on chromosome 13q (Wooster et al., 1994) have opened up a number of research opportunities.

As detailed in the Technical Objectives portion of the proposal, our goal originally was to map a second susceptibility locus for breast and ovarian cancer, using families which were unlinked to BRCA1. This strategy was enhanced by our finding that families which contained a case of male breast cancer were very likely to be not due to BRCA1 (Stratton et al., 1994), and were presumed to be due in large part to this additional locus. This strategy, combined with a total genome search conducted in Dr. Goldgar's laboratory in Utah and Dr. Stratton's lab in the UK, resulted in the localization of BRCA2 to chromosome 13q12-13 at approximately the same time as the present grant was awarded (Wooster et al., 1994).

The first three technical objectives of our original proposal were accomplished early on. Therefore, we have redefined the goals of this proposal to pursue several research goals related to the BRCA2 gene as follows:

1. We will construct fine-scale genetic and physical maps in the BRCA2 region, using both published markers, and STR markers to be developed in our laboratory.

2. We will continue to ascertain families with high incidence of early onset breast cancer for linkage testing. To be eligible, families will have at least four cases of breast cancer diagnosed before age 60. Families which show linkage (or mutation) to BRCA1 and families which appear not to be due to either BRCA1 or BRCA2, will become

part of ongoing separate studies, while families which show evidence of linkage to BRCA2 will be used in revised technical objectives 3-5 of the current proposal.

3. We will test the markers identified in aim 1 above in BRCA2-linked families collected by ourselves and our collaborators which contain key recombinant individuals in order to identify the closest flanking markers for each gene identified. In this way we will localize the susceptibility locus as precisely as possible.

4. We will study large BRCA2 linked families with known ascertainment in order to estimate age and site specific cancer risks. These studies will include prospective follow-up of gene carriers identified through linkage analysis.

5. Using the markers identified in objective 1. above, we will construct 12-locus haplotypes for each of a large set of families which have a high likelihood of being due to BRCA2. Analysis of these haplotypes could narrow the region containing BRCA2 and identify families which may be caused by identical BRCA2 mutations.

Body of Report - Results to date

Creation of STR markers spanning the BRCA2 region

We have thus far identified and developed 8 short tandem repeat (STR) markers in the BRCA2 region, six dinucleotide repeats and two tetranucleotide repeats. New STR markers developed in our laboratory were genotyped on 40 CEPH independent individuals in order to obtain the heterozygosity, number of alleles, and allele frequencies. These STR markers are listed in order along the chromosome in table 1, along with published markers in the region for reference. Six of these eight markers are extremely polymorphic with heterozygosities ranging from 0.78 - 0.88. These, in particular, will be very useful for haplotype generation and comparison,and for identification of key recombinants in families as was done by our group for BRCA1 (Neuhausen et al., 1994).

Ascertainment of families

Families will be ascertained from the Utah Population Database (UPDB), Family Cancer Clinics in the US and in the UK, and by physician and self-referral.

All new breast cancer cases will be identified in the UPDB. All possible genetic relationships between the affected individuals will be calculated. Sets of the largest, most closely related sets of affected individuals will be selected for study. In order to maximize the amount of information for linkage, informative pedigree members will be sampled for DNA.

Pathology blocks will be obtained where possible for deceased cases. For families ascertained from the UPDB, the Utah Cancer Registry will contact each proband for permission to study their family. After permission is obtained, an introductory letter will be sent to each proband in each kindred. A follow-up phone call to establish contact with the proband will be made by the clinic coordinator at which time participation will be discussed and appointments scheduled, family history will be gathered and necessary demographic information obtained.

Female subjects (both affected with breast cancer and unaffected) who are at risk for inheriting a breast cancer susceptibility gene in each family will be administered a short questionnaire on reproductive, demographic, and medical history. Similar procedures will be followed on families ascertained by the other modes. A list of the Utah kindreds enrolled in the study thus far is provided in table 2.

Penetrance Analysis

The study of penetrance in existing families will be confined to families which have posterior probabilities of being due to BRCA2 (posterior probability >0.98), and have a large proportion of individuals available for study. We have successfully used this approach in a set of large Utah kindreds linked to BRCA1 (Goldgar et al., 1994; Goldgar et al., in press; Narod et al., in press).

Currently, we are performing these studies on four Utah families which have sufficient linkage evidence to BRCA2 to qualify for the penetrance/follow-up studies (K107, K2327, K1018, K2044). There are also three kindreds which have been ascertained by the ICR group (F186, F120, F007) which are large enough for these studies. To provide unbiased estimates of penetrance, it will be important to expand the families with regard to the genotype (mutation, or linked haplotype) rather than the phenotype (presence of cancer).

The analyses of penetrance will be done both by using standard life-table estimates of gene carriers, or by using all pedigree information and maximizing the lod-score over the possible age-specific relative risks due to the gene. Potential risk modifiers such as parity and age at first and last pregnancy, oral contraceptive use, etc. will be incorporated into the models to test for interaction with the genetic effects in altering the cancer risks.

We have started this effort by analyzing two large families, one in Utah and the other an Irish family studied by the ICR group. A detailed description of the Utah family, kindred 107 which has been prospectively followed for 45 years, is reported in Goldgar et al. (1995). These two

families (K107, K186) each have clear evidence of linkage to BRCA2 markers (multipoint LOD scores >3.0), a known ascertainment with prospective follow-up, and thus provide an ideal resource for estimation of BRCA2 penetrance.

Statistical analysis of these two families (Easton et al., submitted), yielded the age specific risks of breast cancer as shown in table 2.

As seen in this table, BRCA2 carriers have an estimated risk for female breast cancer of 60% by age 50 and 80% by age 70, and a risk of male breast cancer by age 70 of 6%. A significant excess of ovarian cancer, laryngeal cancer, and prostate cancer was found among BRCA2 carriers(or likely carriers) in these two families.

Future work in this phase of the study will concentrate on adding additional families and extending the penetrance analyses to examine the effect, if any, of the risk factors.

Haplotype analysis

Haplotypes were constructed for 18 families with a history of early-onset breast cancer and which have a high probability (>0.80) of being linked to BRCA2 based on phenotypic and linkage analysis. Currently, ten STRs have been used to construct the haplotypes, with six of them developed by us during the development of a physical map of the BRCA2 region. The STRs utilized are described in Table 1.

Initial results among the collected families indicate minimal haplotype sharing (Table 4). Kindreds 2027 and 8001 share a 4-allele haplotype, but it is comprised of common alleles. If, as seen in other studies, haplotype sharing reflects common BRCA2 mutations, there will be a large number of mutations as represented by these families and haplotypes. The haplotypes identified here will be compared to the haplotypes of the male breast cancer cases.

Conclusions / Future Work

We feel that the results presented on the preceding pages demonstrate that we have made substantial progress toward the revised aims of this grant. It is anticipated that within the upcoming year of this grant, that the BRCA2 gene will be isolated, either by this group or our collaborator on this grant, Dr. Stratton. This will open up additional opportunities in research, through the ability to detect mutations in small families and/or well-defined cases series.

Studies examining correlation between mutation and phenotype can be done to determine if certain mutations give a particularly high risk of breast cancer in males. We do not,however, know with certainty when BRCA2 will be cloned. It is our intent, therefore, to continue with family-based studies as outlined in this proposal.

Specifically, we plan to identify another 10 large families which show linkage to BRCA2 and to begin studies designed to look for the interaction of BRCA2 with known environmental and other genetic risk factors in modulating cancer risk. At the same time we will increase our knowledge of the phenotypic spectrum of BRCA2 mutations, particularly with regard to the incidence of other cancers.

In summary, we are looking ahead to an exciting and productive year of research on this proposal.

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Table 1. STRs for haplotype analysis. The STRs are listed in order from proximal (centromere) to distal (telomere) at 13q12-13. BRCA2 is within this interval. The observed heterozygosity, number of alleles and allele frequencies are based on the CEPH reference panel.

STR	# chromosomes	Observed het	#alleles in CEPH ref	Add'l alleles obs. in samples	Three most common alleles and their frequencies*
D13S290	80	0.46	6	0	1 - 0.71; 3,4 - 0.11
C1+	78	0.78	6	0	5 -0.37; 3 - 0.27; 6 - 0.13
tdj3820+	70	0.80	9	3	9 - 0.41; 4 - 0.11; 8 - 0.24
4247+	76	0.89	18	3	12 - 0.12; 11 - 0.09; 10,30 - 0.08
D13S260	80	0.78	9	0	7 - 0.41; 8 - 0.13; 3,6 - 0.11
GA9+	80	0.63	10	3	12 - 0.35; 10 - 0.30; 11 - 0.09
mB561+	74	0.88	8	0	7 - 0.30; 6 - 0.27; 5 - 0.24
D13S171	80	0.72	6	1	8,3 - 0.32; 10 - 0.25
5370-2C+	78	0.79	11	3	6 - 0.37; 5 - 0.23; 1,13 - 0.08
AC6+	80	0.78	6	0	5 - 0.48; 8 - 0.31; 4 - 0.10
D13S310	++	0.70	5	1	4 - 0.41; 5,7 - 0.24
GB10T+	78	0.56	6	0	5 - 0.57;6 - 0.13;4 - 0.12
D13S267	80	0.69	6	0	12 - 0.44; 4 - 0.29; 8 - 0.17

* For STRs designed by us, allele numbers were assigned based on size from largest to smallest such that even though only 10 alleles were observed for GA9, allele designations are to 14 (no alleles 1,2,6, or 8 were observed). Allele frequencies for D13S markers are from published reports, although the allele numbers have been changed to reflect our numbering system. CEPH 1347-02 was used as a standard to confirm size and allele number designations.

+ STRs developed by us and mapped on a contig comprised of YACs, P1s, BACs, and PACs.

++ CEPH grandparents were used.

Table 2. Description of BRCA2 Family Set.

Kindred	Number of Cancer Cases[1] FBR	MBR	OV	LOD	Posterior Probability[2]
107*	22	3	2	5.06	1.00
8001	0	3	0	n.d.	0.90
8004	1	2	0	n.d.	0.90
2044*	8	1	4	2.13	1.00
2043*	2	1	1	0.86	0.98
2018	3	1	0	n.d.	0.90
937	3	1	0	n.d.	0.90
1018*	9	1	0	2.47	1.00
2328	11	1	0	0.42	0.96
2263	2	1	0	n.d.	0.90
8002	2	1	0	n.d.	0.90
8003	2	1	0	n.d.	0.90
2367	6	0	1	0.40	0.85
2388	3	0	1	0.92	0.95
2027*	4	0	0	0.39	0.85
4328	4	0	0	0.44	0.87
2355	3	0	0	0.36	0.84
2327	11	0	0	1.92	0.99

*Families reported in Wooster et al (1994)
n.d. = Not determined
[1]Excludes cases known to be sporadic (i.e. do not share the BRCA2 haplotype segregating in the family)
FBR =female breast cancer under 60 years MBR = male breast cancer
OV=ovarian cancer
[2]Posterior probability assumes that, *a priori,* 90% of families with male breast and early onset female breast cancers that are unlinked to BRCA1 are due to BRCA2, and 70% of female breast cancer families unlinked to BRCA1 are due to BRCA2.

Table 3. Estimated cumulative risks of breast cancer in BRCA2 carriers.

Cumulative risk Breast by age	Female Breast Cancer K107	K186	Combined	Male Cancer
30	.012	.015	.013	- - - -
40	.12	.15	.13	.0008
50	.59	.61	.60	.008
60	.68	.79	.71	.029
70	.80	.80	.80	.063

Table 4. Haplotype data for the 18 families shown in table 3. Entries in table correspond to alleles (see table 1 for allele frequencies) associated with each haplotype for the given markers.

	STRs examined									
tdj Kindred 267	3820	D13S 4247	260	GA9	mB 561	D13S 171	5370-2C	D13S AC6	D13S 310	
107*	8	28	4	10	8	3	2	6	4	12
8001	8	30	6	10	7	10	5	5	5	4
8004	9	11	4	4	7	8	6	8	4	12
2044*	9	12	10	7	5	9	6	5	4	8
2043*	6	30	3	12	7	10	5	8	4	12
2018	9	12	7	3	8	3	6	6	5	8
937	8	10	4	-	-	8	10	6	7	7
1018*	6	17	8	10	5	8	2	5	4	8
2328	9	10	3	10	5	8	5	5	7	12
2263	9	28	8	-	8	4	-	-	7	12
8002	3	29	7	10	5	8	5	5	5	8
8003	4	12	6	10	6	3	4	5	4	8
2367	6	28	7	10	12	3	7	5	5	4
2388	8	16	7	12	4	10	4	5	5	12
2027*	4	11	3	10	7	10	5	6	7	12
4328	9	10	8	4	8	3	7	8	5	12
2355	9	10	6	4	6	3	7	3	5	8
2327	3	12	2	9	5	10	5	5	3	4